

Current Best Practices for Respiratory Virus Testing

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Diagnostic testing for respiratory viruses has been revolutionized by recent advances that have made rapid and highly accurate tests accessible to clinical laboratories, and it is important that these improved methods be utilized. Accurate detection of respiratory viruses is important in patient care, as it guides both therapy and infection control measures. On a larger scale, the CDC and its collaborating laboratories collect both data and isolates from clinical laboratories for national surveillance, and the use of high-quality tests in clinical laboratories can improve the quality of these data.

In the past decade, there has been a marked improvement in the availability of laboratory and point-of-care tests for the diagnosis of respiratory virus infections. Commercial manufacturers have introduced new rapid respiratory virus culture methods, pooled antibody reagents, rapid antigen direct tests (RADTs), and improved specimen collection devices. Most important among these is the development of commercial, Food and Drug Administration (FDA)-approved, and laboratory-developed nucleic acid amplification tests (NAATs). The introduction of these new systems has created new challenges for laboratory directors, who must decide which of the many tests to offer and what specimen types to accept for diagnostic testing.

This working group considered several current issues in diagnostic testing for respiratory viruses, including the best methods for detection of respiratory viruses, sample handling, turnaround time, the scope and seasonal use of tests, testing for antiviral resistance, and monitoring the performance of diagnostic tests. Our goals in discussing these issues were, when possible, to come to a consensus on the best practices and to raise questions for further consideration and investigation.

METHODS FOR THE DETECTION OF RESPIRATORY VIRUSES

There was consensus among the group members that the use of NAATs in the routine clinical setting has dramatically changed our approach to the diagnosis of viral respiratory tract infections. Traditional virus detection methods, including RADTs, direct fluorescent antibody testing (DFA), and virus culture, can be effective diagnostic tools but are often inferior in assay sensitivity, specificity, time to virus identification, and breadth of pathogen detection compared to NAATs (30).

The times to results that are possible with these tests vary widely, as do the viruses that they can detect. RADTs are simple to perform and provide results within 15 to 30 min but

are limited to the detection of influenza A virus, influenza B virus, and respiratory syncytial virus (RSV) (30, 46, 51). DFA can be performed in as little as 30 to 60 min and can detect 8 of the common respiratory viruses (adenovirus; influenza A virus; influenza B virus; human metapneumovirus [hMPV]; parainfluenza virus types 1, 2, and 3 [PIV-1, PIV-2, and PIV-3]; and RSV) (26). Rapid cell culture (shell vial or cluster trays) can detect adenovirus, influenza A virus, influenza B virus, PIV-1, PIV-2, PIV-3, hMPV, and RSV (35). Traditional tube cell culture can have a broader scope of pathogen detection depending on the cell lines used. However, it usually takes 3 to 7 days to detect these viruses by traditional tube culture, whereas rapid cell culture can generally detect >90% of the viruses within 48 h (7). DFA and rapid cell culture methods can therefore provide results within a time frame that could affect patient management if testing is performed locally. However, outside larger hospitals and reference laboratories, DFA and rapid cell culture are generally not available, and the results vary and can be delayed depending on virus viability and the transit time to a reference laboratory.

The accuracy of these different tests also varies widely. The utility of RADTs is greatly limited by their modest sensitivities (6, 11, 15, 16, 24, 30, 45). The sensitivity of these tests for influenza viruses and RSV are 10 to 85% and 50 to 98%, respectively (reference 30 and references therein). The specificities of RADTs are generally reported to be high (6, 11, 15, 16, 24, 30, 45), but a recent report suggests that this might be incorrect, and this warrants further investigation (44). Although the specificities of DFA and rapid cell culture are high, the sensitivities of the tests vary by virus (and sometimes by viral strain) from a low of 50% (adenovirus DFA and RSV culture) to a high of >80% (RSV DFA and influenza A virus culture) compared to NAATs (26, 30, 31, 35, 52). In addition, although the 8 viruses commonly detected are responsible for a large portion of viral respiratory tract infections, select coronaviruses (229E, OC43, NL63, and HKU-1), parainfluenza virus type 4, rhinovirus, and potentially bocavirus are also significant causes of respiratory disease and are generally only detected using NAATs (4). For example, studies performed during the height of the New York City outbreak of the 2009 influenza A virus H1N1 pandemic demonstrated that the overall rates of positivity for any respiratory virus in the clinical

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samples were highly dependent on the test methodology and the number of viral targets detected by the assay (16). A single virus or multiple viruses (4% of the samples) were detected with prevalence rates of approximately 20% for RADTs (3 viruses), 20% for DFA (8 viruses), 35% for rapid cell culture (7 viruses), and 63% for a comprehensive NAAT (15 viruses).

THE ROLES OF RADTs, DFA, AND VIRAL CULTURE IN THE AGE OF NAATs

Knowing the caveats of traditional testing compared to NAATs, the question as to whether there is still a role for RADTs, DFA, and culture arises. The working group considered this question carefully, and it is the recommendation of the panel members that RADTs should be replaced by more sensitive NAATs whenever practical. Until FDA-cleared, easy-to-use, cartridge-based NAATs ("molecular point-of-care tests") become available, the participants acknowledge that for most community hospitals and physician's offices, RADTs may be the only diagnostic method available. The opinions of the participants of the working group differed about how forceful laboratory directors should be in their efforts to eliminate the use of RADTs, but there was consensus that it is not possible to discontinue their use at all institutions. Laboratory directors, in conjunction with infectious-disease physicians and infection control practitioners, need to play a key role in educating their medical staffs on the limitations of RADTs (low sensitivity and marginal specificity) and to caution them on the clinical and infection control consequences of a missed diagnosis due to a negative RADT. Though the cost per test for NAATs may be significantly higher, the ease of use and the high quality and importance of the result, particularly for the inpatient setting, need to be considered and weighed against the total cost of patient care. These costs include inappropriate antibiotic use (cost and increasing drug resistance), increased length of stay with decreased reimbursement, increased ancillary testing due to the lack of a diagnosis, and potential for nosocomial outbreaks with significant morbidity and mortality (25, 28). At this time, the combination of RADT or DFA with recourse to rapid viral culture for RADT- or DFA-negative samples may be sufficient for respiratory infections that are not severe, such as those in patients who are cared for in the outpatient setting. DFA would be applicable as a rapid screening test for hospitalized patients, with additional comprehensive NAAT for specimens that are negative by DFA. The utility of rapid viral culture is limited by the range of viruses detected, variable viability of the viruses during transport and storage, and inability to easily detect mixed infections. In addition, although rapid virus culture is faster than conventional tube culture, it is still generally slower than RADT, DFA, and NAAT.

Studies have demonstrated that mixed viral infections are detected in 3.3 to 30.0% of samples when a wide variety of viral respiratory pathogens are included in the testing (4, 16, 39, 41). Although the significance of mixed viral infections still needs to be clearly defined, the clinical impact in critically ill patients and patients with comorbidities or immune suppression must be considered potentially severe. During the height of influenza season, when patient cohorting is often necessary due to large numbers of patients with respiratory illness, the consequences of a second viral infection in an already seriously ill

hospitalized patient could be substantial. In addition, with the advent of new antiviral agents, such as those targeted to the treatment of severe rhinovirus infections, comprehensive targeted diagnostics will become increasingly important (42, 54).

The working group agreed that traditional tube culture, although generally too slow to impact patient management, is still applicable in certain situations, including the care of immunocompromised patients, where additional viruses, such as herpes simplex virus types 1 and 2 and cytomegalovirus, can play a key role. These viruses are not yet included in standard NAAT panels for respiratory virus detection. Other situations where culture still plays an important role are in providing clinical isolates for epidemiology studies, for the establishment of vaccine candidates, to evaluate mechanisms of antiviral resistance for new antiviral drugs, for clinical trial studies, and to identify potential novel viral agents. Certain viruses, such as adenovirus, that demonstrate significant divergence, can be a challenge for detection by NAATs, and culture may be necessary to supplement NAATs (40).

APPROPRIATE SAMPLE COLLECTION, TRANSPORT, AND STORAGE

Respiratory virus detection is highly dependent on the type of sample collected, the time of collection after the onset of clinical symptoms, the age of the patient, and the transport and storage of the sample prior to testing (48, 49). Several different upper respiratory tract specimens are applicable for testing, including nasopharyngeal (NP) washes, NP aspirates, and NP swabs placed in virus transport media (48, 49). There are limited data that support the use of combined nose-throat swabs for influenza A virus testing by NAAT (14). Detection of 12 respiratory viruses using a NAAT panel was significantly less sensitive with oropharyngeal swab specimens (54.2%) than with either nasopharyngeal swabs (73.3%) or nasopharyngeal wash specimens (84.9%) (33). This may be due to the substantially lower viral loads in the oropharynx than in the nasopharynx (23).

There was consensus among the members of the working group that use of NP swabs for specimen collection is an important advance in testing for respiratory viruses. NP flocked swabs (Copan, Brescia, Italy) have generally been found to yield specimens as good as nasopharyngeal wash specimens for detection of respiratory viruses by NAAT or DFA (1, 50). This is presumably because flocked swabs effectively collect and release respiratory epithelial cells from NP specimens (12). NP flocked swabs are generally easier to collect from adult patients and older children than NP aspirates or washes. For safety reasons, the collection of an NP aspirate may be indicated in young children. The use of nasal or oropharyngeal swab samples is an area of active investigation, but routine use of these specimens is not recommended at present because of concerns about sensitivity for virus detection in some studies (32, 38). Lower respiratory tract samples, such as induced sputum, protected brush samples, and bronchial alveolar lavage (BAL) samples, may be necessary, as several studies have demonstrated that in some severe cases of influenza, upper respiratory tract samples are negative while lower respiratory tract samples are positive (29, 37, 53). For optimal results, samples should be collected within 3 to 5 days of

symptom onset, transported to the laboratory on wet ice, and refrigerated (2 to 8°C) if testing is to be performed within 48 h (10, 48, 49). If testing is delayed, the samples should be stored frozen at -80°C (48, 49). Multiple freeze-thaws are not recommended, as this process decreases the viral titer, particularly for culture-based methods. In general, methods tend to perform better with pediatric samples, since children shed higher titers of virus and for longer periods than adults (2, 8, 20, 27).

TEST TURNAROUND TIMES

Testing needs to be completed within a time frame that can affect patient management (i.e., initiation or discontinuation of antivirals and antibiotics and supportive care); provide for efficient bed utilization and appropriate infection control measures, such as cohorting, to reduce nosocomial transmission; and identify outbreak situations. Therefore, batch testing performed 2 to 3 times a week is not optimal. It is recommended by the working group that results of NAATs, RADTs, and DFA tests be available within 24 h of sample collection, although the committee acknowledges that this may not be possible for all laboratories, especially during times when staffing is limited, such as weekends and holidays.

SCOPE OF RESPIRATORY VIRUS TESTING

Should laboratories screen for all respiratory viruses, at all times of the year, and in all patient populations? Many respiratory viruses demonstrate seasonal variations in prevalence, particularly in temperate areas. For example, RSV is generally detected from November through March, influenza virus between December and April, and hMPV between December and May in the United States. Often, laboratories restrict testing for specific respiratory viruses during certain seasons. This type of restriction is especially indicated for tests with lower specificity (perhaps the RADTs) outside the influenza or RSV seasons, as the positive predictive value is greatly diminished when the prevalence of these viral infections is low. However, with global travel, many "seasonal" viruses are now detected throughout the year. Therefore, limiting detection to seasonal periods can result in missing an important outbreak, such as the 2009 influenza A virus H1N1 pandemic in the United States, which began at the end of the "traditional flu season" (April 2009) and continued throughout the summer months. Since NAATs and viral culture are highly specific, the positive predictive value of these tests remains high during times of low viral prevalence. However, laboratory directors should consider confirmatory testing of positive results when a virus is detected during an unusual period. Laboratories should monitor the local seasonal prevalence of the viruses routinely screened for and make testing decisions based upon these data and additional resources and epidemiology information provided on the CDC website (<http://www.cdc.gov/flu/>). Laboratories must always keep in mind that testing algorithms must be adaptable to unexpected local, national, and international events, such as the 2009 influenza A virus H1N1 pandemic.

The working group agreed that limiting testing for routine respiratory viruses to certain patient populations, such as children, is not the best clinical practice. For example, although RSV and hMPV are primarily detected in pediatric samples

(19, 22, 34), severe RSV and hMPV infections have been described in adults of all ages with and without underlying disease, such as chronic obstructive pulmonary disease (COPD) or asthma (19, 21, 22). In addition, infections with these pathogens can have atypical presentations, such as hMPV pericarditis in an otherwise healthy adult (13). Therefore, age may help triage initial testing but should not govern the final range of viruses included in diagnostic testing algorithms.

TESTING FOR ANTIVIRAL RESISTANCE

Currently, FDA-approved antiviral therapeutic agents for respiratory viral infections are limited to the treatment of influenza. The classes of influenza antivirals include the adamantanes (amantadine and rimantadine) and the neuraminidase inhibitors (oseltamivir and zanamivir) (17). The efficacy of aerosolized ribavirin for treatment of influenza is not well understood, and this is not an FDA-approved use for the antiviral (43). The susceptibility of influenza virus subtypes to FDA-approved antivirals varies by antiviral, with seasonal influenza A virus H1N1 being susceptible to the adamantanes and zanamivir and >99% resistant to oseltamivir and seasonal influenza A virus H3N2, 2009 influenza A virus H1N1, and influenza B virus being susceptible to oseltamivir and zanamivir and 100% resistant to the adamantanes (17, 18, 47). During the course of the 2009 influenza A virus H1N1 pandemic, cases were reported in which patients with underlying disease developed oseltamivir resistance during prolonged treatment (3). Limited nosocomial transmission of oseltamivir-resistant 2009 influenza A virus H1N1 has occurred in immunocompromised patients (9, 36). The need for routine influenza virus antiviral resistance testing therefore depends on the circulating strains and known resistance patterns (17, 18, 47). During the 2010–2011 influenza season, routine resistance testing was not indicated, as the antiviral susceptibility patterns were the same for the two common subtypes of circulating influenza A virus. During the 2009–2010 influenza season, varying susceptibilities to antivirals among circulating subtypes of influenza A virus meant that antiviral susceptibility testing was needed and that subtyping could be used as a guide for selecting appropriate antiviral therapy. In the event of a shift in circulating strains or increasing resistance noted for a strain, the use of antiviral resistance testing should be reevaluated and may be indicated for patients at high risk for severe disease.

MONITORING THE PERFORMANCE OF DIAGNOSTIC TESTS

Early in the course of the 2009 influenza A virus H1N1 pandemic, several studies showed that the performances of the RADTs, DFA, and viral culture were suboptimal (5, 16, 24, 45). The working group discussed the potential reasons for this decline in test performance. The reasons for the decline in test performance, compared to both previously published peer-reviewed articles and manufacturers' claims, as determined in tightly controlled clinical trials, include (i) comparison of RADTs, DFA, and culture performance to NAAT, the new, more sensitive "gold standard"; (ii) testing of patients late in the course of an infection, when they are shedding virus at levels below the detection limit of RADTs, DFA, or culture

but still detectable by NAAT; (iii) testing the “worried well”; (iv) improper collection, storage, and transport of samples; and (v) performance of testing by untrained or poorly supervised personnel, especially in the case of Clinical Laboratory Improvement Act (CLIA) waived tests. In addition, antigenic divergence of new circulating strains can lead to a decrease in detection by tests that rely on antibody interactions with specific viral epitopes for primary detection (RADTs and DFA) or for culture confirmation. Similarly, new RNA or DNA sequence variants can affect the performance of NAATs due to primer and/or probe mismatches. Once tests are FDA cleared/approved, manufacturers are not under an obligation to change test components or monitor test performance. Therefore, assay performance can significantly decline over time without the knowledge of the user. It is the responsibility of laboratories to continually assess the performance of their tests and, when a decline in performance is noted, investigate possible on-site causes of poor performance, compare alternative tests, and notify the manufacturer and, finally, the FDA if the changes are significant enough to potentially cause clinical harm. The participants strongly feel that an FDA reevaluation process should be in place whereby manufacturers can change primers and/or probes to accommodate genetic variants in a simple yet safe way without the necessity to perform full clinical trials that can take a year to complete.

CONCLUSIONS AND FUTURE CONSIDERATIONS

It is increasingly evident that NAATs are superior to traditional virus detection methods due to enhanced sensitivity and specificity, a broad range of virus detection, and rapid turnaround time. However, until such time as easy-to-use point-of-care NAATs are available, many laboratories will need to continue using RADTs or other methods. Several questions remain unanswered about the use of NAATs, and the participants in the working group agreed that they are important areas for future investigation. What are the appropriate test panels? Should NAATs detect only the common respiratory viruses or those for which there are therapeutic options? Although therapeutic options may not be available for many viruses, the risk of nosocomial spread in health care institutions cannot be dismissed. Is it better to use mix-and-match panels, or is a single comprehensive panel best? How do we interpret mixed infections, and what is the relevance of detecting a virus in an asymptomatic patient? Finally, how do we work with the FDA to establish a safe yet fast way to modify FDA-cleared/approved tests so that optimal reactivity and detection can be maintained as viral strains shift? As clinical microbiologists, our task is to provide the most clinically relevant diagnostic information, and recent improvements in testing for respiratory viruses support us in this task.

Session discussants: Matthew J. Binnicker, Paul Bourbeau, Lynn Boyer, Paul Compangone, Christian Coogan, Richard Hodinka, Mark LaRocco, Duane W. Newton, Ribhi Shawar, Gongyi Shi, B. Jane Smith, Richard B. Thomson, and Hemant C. Vaidya.

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